JOURNAL OF LABELLED COMPOUNDS AND RADIOPHARMACEUTICALS
J Label Compd Radiopharm 2004; **47**: 217–232.
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jlcr.816

Research Article

Substitution-reduction: an alternative process for the [^{18}F]*N*-(2-fluoroethylation) of anilines

Emmanuelle Briard* and Victor W. Pike
PET Radiopharmaceutical Sciences, Molecular Imaging Branch, National Institute of Mental Health, National Institutes of Health, Building 10, Rm. B3 C346, 10 Center Drive, Bethesda, MD 20892-1003, USA

Summary

Substitution of a halo atom (chloro or bromo) in easily prepared *N*-haloacetyl-anilines with no-carrier added (NCA) cyclotron-produced [^{18}F]fluoride ion (^{18}F, $t_{1/2}$ = 109.8 min; β^+ = 96.9%), followed by reduction with borane–tetrahydrofuran (BH_3–THF), provides an alternative route to NCA [^{18}F]*N*-(2-fluoroethyl)-anilines. This two-step and one-pot process is rapid (~50 min) and moderately high yielding (~40% decay-corrected radiochemical yield (RCY) overall). In the nucleophilic substitution reaction, 18-crown-6 is preferred to Kryptofix® 222 as complexing agent for the solubilization of the counter-ion (K^+), derived from an added metal salt, in acetonitrile. Weakly basic potassium bicarbonate is preferred as the added metal salt. Inclusion of a small amount of water, equating to 4–5 molar equivalents relative to 18-crown-6, base or precursor (held in equimolar ratio), is beneficial in preventing the adsorption of radioactivity onto the wall of the glass reaction vessel and for achieving high RCY in the nucleophilic substitution reaction. BH_3–THF is effective for the rapid reduction of the generated [^{18}F]*N*-fluoroacetyl-aniline to the [^{18}F]*N*-(2-fluoroethyl)-aniline. Copyright © 2004 John Wiley & Sons, Ltd.

Key Words: fluorine-18; [^{18}F]*N*-(2-fluoroethylation); anilines; *N*-haloacetyl-anilines; nucleophilic substitution; reduction

Introduction

Fluorine-18 ($t_{1/2}$ = 109.8 min; β^+ = 96.9%) is a major positron-emitter for labeling tracers for application in clinical research[1–3] and drug development[4,5] with positron emission tomography (PET).[1–3] The usual source of no-carrier-

*Correspondence to: E. Briard, PET Radiopharmaceutical Sciences, Molecular Imaging Branch, National Institute of Mental Health, National Institutes of Health, Building 10, Room B3 C346, 10 Center Drive, Bethesda, MD 20892-1003, USA. E-mail: briarde@intra.nimh.nih.gov

Received 25 November 2003
Revised 20 January 2004
Accepted 22 January 2004

added (NCA) fluorine-18 is the $^{18}O(p,n)^{18}F$ reaction on ^{18}O-enriched water, which provides aqueous NCA [^{18}F]fluoride ion.[6] Invariably, the use of [^{18}F]fluoride ion for labeling tracers must proceed with a nucleophilic substitution reaction, either directly on a precursor or on another species that can then be used as a labeling agent.[7] Important amongst such labeling agents are compounds of formula $X(CH_2)_n^{18}F$, where n may range from 1 to 4 and X may be halogen (Cl, Br or I),[8–20] alkyl sulfonyloxy (OMs or OTf)[11,18,19,21] or aryl sulfonyloxy (OTs).[10,11,16,20,22] These labeling agents are usually prepared from the corresponding compounds with formula $X(CH_2)_nX$ and are often used to perform [^{18}F]ω-fluoroalkylations at nucleophilic centers (e.g. OH, NH_2, SH).[7] This two-step process has given rise to several useful radiotracers, including [^{18}F]*N*-(2-fluoroethyl)-spiperone,[23–25] [^{18}F]FECNT[26,27] and [^{18}F]*O*-(2-fluoroethyl)-L-tyrosine.[28]

[^{18}F](2-Fluoroethylation) ($n = 2$) at a hetero atom Y (Y = N(H/R), O or S) has been especially popular, because it provides a convenient means to label organic molecules, often with little change to their lipophilicity, pharmacology and pharmacokinetics. Acceptable radiochemical yields can be obtained from the reactions of these labeling agents with good nucleophiles (e.g. phenoxides,[11,19,28] secondary aliphatic amines[16,19,21,26] and carboxylates[20]). However, there are few examples of this process being useful for the [^{18}F](2-fluoroethylation) of weak nucleophiles (e.g. aromatic amines[29,30]).

An alternative means for creating an [^{18}F]2-fluoroethyl group at a hetero atom is to directly substitute X in $RY(CH_2)_2X$ with [^{18}F]fluoride ion.[25,31,32] However, this single step process is sometimes low yielding. Also the precursor $RY(CH_2)_2X$ may be difficult to prepare or vulnerable, for example, to elimination, hydrolysis or, in the case of Y = N(H/R), formation of an aziridinum intermediate.[21,33]

In our recent experience the special case of direct [^{18}F]*N*-2-fluoroethylation of anilines is quite difficult to achieve.[30] Here we report an alternative two-step process for the synthesis of [^{18}F]*N*-(2-fluoroethyl)-anilines based on direct nucleophilic substitution with NCA [^{18}F]fluoride ion in an *N*-haloacetyl-aniline (halo = Cl or Br) followed by rapid reduction of the generated [^{18}F]*N*-fluoroacetyl-aniline *in situ*.

Results and discussion

The reference *N*-(2-fluoroethyl)-anilines **3a** and **3b** were prepared in three steps from aniline and *N*-methyl-aniline, respectively (Figure 1). First the precursor *N*-bromoacetyl- and *N*-chloroacetyl-anilines **1a–d** were obtained in high yields by treating the aniline with the corresponding haloacetic anhydride in glacial acetic acid.[34] **1a–1d** were converted into the corresponding *N*-fluoroacetyl-anilines **2a** and **2b** in moderate yield by treatment with powdered potassium

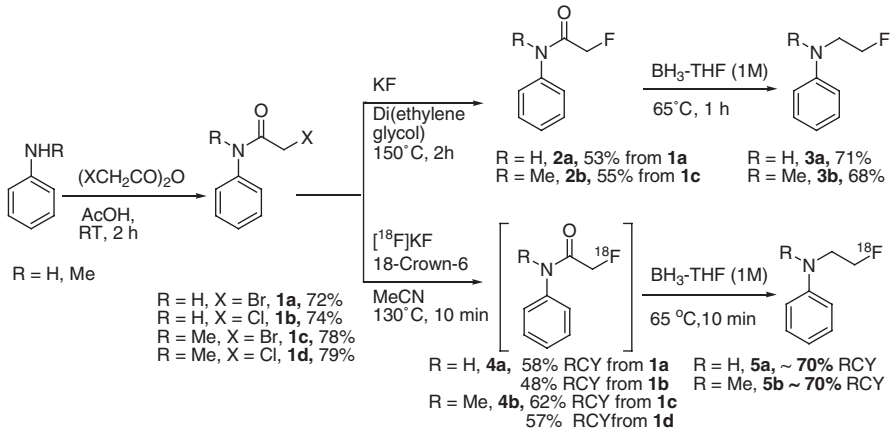

Figure 1. Synthesis of *N*-(2-fluoroethyl)-anilines and [^{18}F]*N*-(2-fluoroethyl)-anilines

fluoride in di(ethylene glycol).[35,†] Treatment of **2a** and **2b** with borane-tetrahydrofuran complex[36] (BH_3–THF) gave **3a** and **3b**, respectively, in moderately high yield.

In this study, we used *N*-bromoacetyl-aniline **1a** as a model substrate for optimizing the nucleophilic substitution of the bromo atom by NCA [^{18}F]fluoride ion. Complexing agent, amount of added water, metal salt, amount of precursor, temperature, reaction time, leaving group and *N*-substitution were varied. Favorable conditions were then applied to the chloro analog **1b** and the secondary amides **1c** and **1d**. Conditions were also established for the reduction of the [^{18}F]*N*-fluoroacetyl-anilines **4a** and **4b** to the desired [^{18}F]*N*-(2-fluoroethyl)-anilines **5a** and **5b**.

Optimization of nucleophilic substitution with NCA [^{18}F]fluoride ion

Choice of complexing agent. Initially, radiolabeling was tried with **1a** (5 mg) under conditions widely applied to achieve successful aliphatic nucleophilic substitution reactions with NCA [^{18}F]fluoride ion, namely using acetonitrile as solvent, Kryptofix® 222 as cryptand and potassium carbonate as added metal salt.[37] The decay-corrected radiochemical yield (RCY) of [^{18}F]*N*-fluoroacetyl-aniline **4a** from a reaction performed in a glass vessel heated in an oil bath at 130°C for 10 min was 15%. In reactions where the amount of precursor was increased, RCYs were even lower. The *N*-bromoacetyl compound **1a** was found to be capable of *N*-alkylating Kryptofix 222 under the reaction

† Fluoroacetyl chloride is no longer commercially available and hence it was not possible to prepare **2a** and **2b** by single step acylation of the anilines.

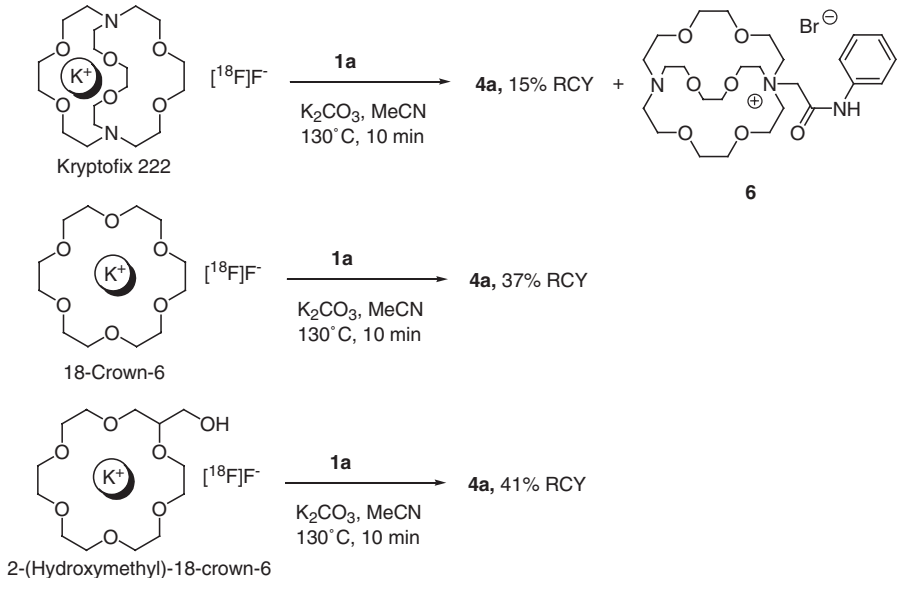

Figure 2. Effect of complexing agent on the radiofluoridation of 1a

conditions, as evidenced by new ^{1}H- and ^{13}C-NMR signals after heating Kryptofix 222 with one molar equivalent of **1a** in CD_3CN at 130°C for 10 min (see Experimental). The mono-alkylated product **6** (Figure 2), was also detected by LC-MS. By switching the complexing agent to the amine-devoid 18-crown-6[38], **4a** was obtained in a much higher RCY (37%). As expected, no reaction between the precursor and 18-crown-6 occurred under these conditions. However, a high proportion (55%) of the radioactivity, assumed to be [^{18}F]fluoride ion, became adhered to the wall of the glass vessel during the course of the reaction and was unavailable for reaction.

The use of 2-(hydroxymethyl)-18-crown-6 as complexing agent was also investigated. Under conditions where the molar ratio of complexing agent, precursor **1a** and potassium ion (as carbonate) was 4:1:1, respectively, [^{18}F]*N*-fluoroacetyl-aniline **4a** was obtained in 41% RCY. Adsorption of radioactivity onto the glass reaction vessel was 31% at the end of the reaction. Evidently, 2-(hydroxymethyl)-18-crown-6 adequately solubilized the K^+ ion in acetonitrile and also promoted the nucleophilicity of the [^{18}F]fluoride ion. This result showed the tolerance of the reaction for the presence of free aliphatic hydroxyl groups.

18-Crown-6 was used as the preferred complexing agent, since it gave higher RCYs than Kryptofix 222 and was cheaper than its 2-hydroxymethyl derivative (or indeed Kryptofix 222).

Effect of added water. The NCA [^{18}F]fluoride ion available for our reactions at the end of azeotropic drying is expected to be almost dehydrated or almost 'naked' i.e. to exist as $F^-(H_2O)_n$ where n is very small. The nucleophilicity of fluoride ion (as R_4N^+ salts) in aliphatic substitution reactions in acetonitrile[39] or aprotic solvents[40] depends on its degree of solvation, such that when $n = 1.5$ reaction rates may be about 100-fold higher than when $n = 8.5$.[40] Some nucleophilicity may be retained when n is as high as 10.0.[39] The *N*-haloacetylanilines **1a–1d** are expected to be very reactive‡ towards nucleophiles by virtue of the expected activating effect of the α-carbonyl group.[41,42] Tolerance for a trace of water in some nucleophilic substitution reactions of [^{18}F]fluoride ion is known.[43,44] Hence, the reactive *N*-haloacetyl anilines might be expected to react with moderately hydrated fluoride ion (i.e. fluoride ion that is moderately nucleophilic).

The adsorption of NCA [^{18}F]fluoride ion from organic solvents to reaction vessel walls is a well-known phenomenon.[45] Adsorption of radioactivity onto the glass reaction vessel wall was very evident in this study. 'Naked' [^{18}F]fluoride ion most probably adsorbs onto the walls of glass vessels through stabilizing interactions (hydrogen-bonding) with surface hydroxyl groups. Strategies for reducing such adsorption have included silylation of glass vessels and the use of vessels with non-hydroxylic surfaces (glassy carbon or platinum).[45] However, these strategies were not explored here.

Instead, we reasoned that addition of a low proportion of water to the NCA [^{18}F]fluoride ion might hydrate it sufficiently to hinder its adsorption onto the glass vessel wall without destroying its nucleophilicity towards the reactive **1a**. Accordingly, we tried reactions of [^{18}F]fluoride ion with **1a–1d** to test this hypothesis. In the presence of a small proportion of added water, the reactions generally proceeded more reproducibly and with less adsorption of radioactivity onto the glass vessel wall. These reactions occurred cleanly. There were no radioactive by-products in the case of the secondary amides **1c** and **1d** (e.g. Figure 3), while the primary amides **1a** and **1b** gave only one minor radioactive by-product (<10% of radioactivity in analyte; HPLC retention time 10.6 min). Unreacted precursor always remained in the reaction mixtures.

For three different amounts of precursor **1a** (2.1, 3.0 and 4.5 mg; 9.8, 14 and 21 μmol), we varied the molar ratio of water while **1a**, complexing agent and metal salt were kept equimolar (Figure 4). In each series, the RCYs were maximal when the molar ratio of water to each of the other components was 4:1. The maximal RCY was highest (58%) when using 3 mg (14 μmol) of **1a** (Figure 4).

The hydrations of 18-crown-6 and its potassium complex have been well studied.[46] A few water molecules of hydration greatly enhance the selectivity

‡The ability of **1a** to alkylate Kryptofix 222 is evidence of this reactivity.

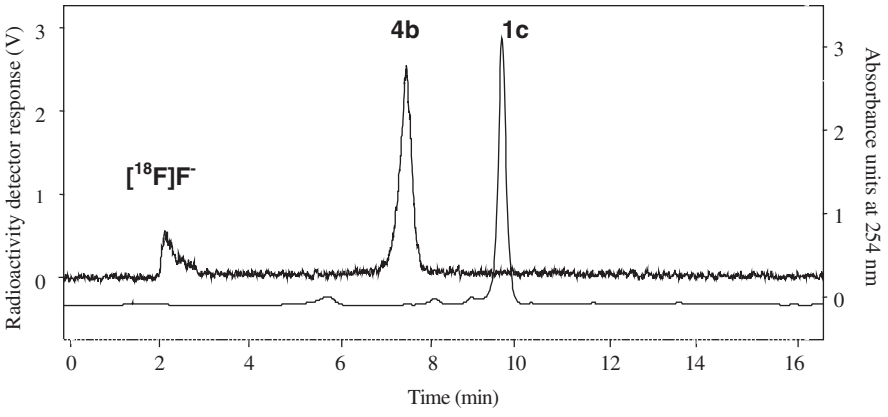

Figure 3. Representative chromatogram from the HPLC analysis of the products from a nucleophilic substitution reaction ([^{18}F]fluoride ion with 1c to give 4b)

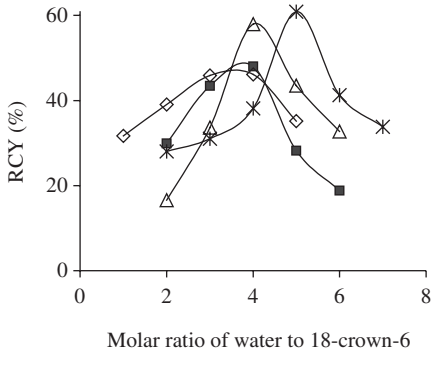

Figure 4. Effect of water on RCY of 4a from 1a treated with [^{18}F]fluoride ion in MeCN (0.65 ml) at 130°C for 10 min. (■) 1a (2.1 mg; 9.8 μmol): 18-crown-6 (9.8 μmol): $KHCO_3$ (9.8 μmol) (△) 1a (3.0 mg; 14 μmol): 18-crown-6 (14 μmol): $KHCO_3$ (14 μmol). (◇) 1a (4.5 mg; 21 μmol): 18-crown-6 (21 μmol): $KHCO_3$ (21 μmol). (✳) 1a (3.0 mg; 14 μmol): 18-crown-6 (9.8 μmol): $KHCO_3$ (9.8 μmol)

of 18-crown-6 for binding potassium ion versus other metal ions.[47] Thus, trace water added to the 18-crown-6-mediated reactions of **1a** with [^{18}F]fluoride ion most probably serves to solubilize and stabilize the 18-crown-6-K^+ complex by hydration. The added water may also help to solubilize anions, principally the bulk of added bicarbonate ion, but also trace fluoride ion, trace reaction product and bromide ion.

Five molar equivalents of water (49 μmol) were necessary to reach a maximal RCY of **4a** of 60%, when using 21 μmol of amide precursor **1a** and

9.8 μmol each of 18-crown-6 and potassium bicarbonate (Figure 4). The requirement for an extra molar equivalent of water under these conditions of increased proportion of precursor **1a** suggests that some of the added water is consumed in hydration of the precursor. This might occur through hydrogen bonding to the amido group.

The sharp decline in RCYs when the molar ratios of water to other components of the reaction increased above 4 or 5 (Figure 4) suggests that under these conditions the [^{18}F]fluoride ion became increasingly hydrated and less nucleophilic.

The inclusion of 4 molar equivalents of water in the reactions limited the adsorption of radioactivity (assumed to be [^{18}F]fluoride ion) onto the glass wall of the reaction vessel to an acceptable level (average ~20%; Table 1, entries 8, 12–14); generally, the level of adsorption decreased with increased proportion of water.

Having established the beneficial effects of an added trace of water on the overall outcome of the nucleophilic substitution reaction, we proceeded to investigate the effect of varying other parameters of the reaction, as follows.

Table 1. Results from the radiofluoridations of *N*-haloacetyl-anilines under various conditions[a]

Entry	Salt	Precursor (♯, mg; μmol)	Temperature (°C)	Time (min)	RCY[b] (%)	RCY[c] (%)	Solubility[d] (%)
1	Cs_2CO_3	**1a**, 3, 14	130	10	7	33	23
2	K_2CO_3	**1a**, 3, 14	130	10	35	57	57
3	KI	**1a**, 3, 14	130	10	37	59	64
4	$KHCO_3$	**1a**, 3, 14	80	10	29	48	61
5	$KHCO_3$	**1a**, 5, 23	80	10	31	44	70
6	$KHCO_3$	**1a**, 7, 33	80	10	43	60	74
7	$KHCO_3$	**1a**, 3, 14	110	10	39	64	67
8	$KHCO_3$	**1a**, 3, 14	130	10	58	72	76
9	$KHCO_3$	**1a**, 3, 14	130	5	44	74	70
10	$KHCO_3$	**1a**, 3, 14	130	20	48	67	72
11	$KHCO_3$	**1a**, 3, 14	130	30	43	58	74
12	$KHCO_3$	**1b**, 3, 18	130	10	48	80	79
13	$KHCO_3$	**1c**, 3, 13	130	10	62	71	87
14	$KHCO_3$	**1d**, 3, 16	130	10	57	76	77

[a]Initials conditions: precursor (as listed), 18-crown-6 (14 μmol), salt (14 μmol in metal ion), H_2O (56 μmol), MeCN (0.65 ml).
[b]RCY from initial activity of NCA [^{18}F]fluoride ion in reaction vessel.
[c]RCY from radioactivity in solution at the end of reaction.
[d]Percent of original radioactivity in solution at the end of reaction; remainder is adhered to the glass reaction vessel wall.

Effect of added metal salt. Reactions of precursor **1a** with [^{18}F]fluoride ion, in which the added metal salt was cesium carbonate, potassium carbonate, potassium iodide or potassium bicarbonate gave **4a** in 7, 35, 37 and 58% RCY, respectively (Table 1, entries 1–3, 8). The low RCY from the use of cesium carbonate is due to the poor solubilization of 'cesium [^{18}F]fluoride' by 18-crown-6; indeed 77% of the radioactivity was adsorbed onto the vessel wall at the end of the reaction (Table 1, entry 1). The higher RCY with potassium bicarbonate is attributed to less decomposition of the reactive precursor under the more weakly basic conditions. Potassium bicarbonate was therefore used as the preferred added metal salt.

Effect of precursor quantity. Increasing the amount of precursor **1a** from 3 mg (14 μmol) to 7 mg (33 μmol) increased the RCY from 29 to 43% for reactions carried out for 10 min in an oil-bath at 80°C (Table 1, entries 4–6). At higher temperature (130°C), 3 mg (14 μmol) of precursor gave the highest RCY (61%) in the precursor range 2.1–4.5 mg (Figure 4). Thus the reaction works with a moderately low amount of precursor, as might be demanded by a need to conserve expensive or rare precursor in radiopharmaceutical production.

Effect of temperature. By increasing the temperature from 80 to 130°C, the RCY of **2a** increased from 29 to 58% (Table 1, entries, 4, 7, 8).

Effect of reaction time. For reactions carried out at 130°C with 3 mg of **1a**, RCYs of **2a** increased to 58% for reaction times up to 10 min and then, because of the formation of radioactive by-products, declined to 43% for reactions up to 30 min (Table 1, entries 8–11).

Effect of leaving group. A bromo leaving group gives the desired [^{18}F]*N*-fluoroacetyl-aniline **4a** or **4b** in appreciably greater RCYs than a chloro leaving group (Table 1, cf. entries 8 with 12 and 13 with 14). The *N*-bromoacetyl-anilines are just as easily prepared as the *N*-chloroacetyl-anilines and are stable to storage. A bromo precursor is therefore preferred.

Effect of N-substitution. In a previous study, where [^{18}F]fluoride ion was used to displace tosyl from *N*-α-tosylmethyl-anilines, it was noted that primary amides gave low RCYs (~6%), whereas their secondary *N*-ethyl amido analogs, gave moderately high RCYs (~60%).[48] This marked difference was attributed to deprotonation of the primary amido group under the basic conditions (Kryptofix 222-K_2CO_3) and its adverse effect on the nucleophilicity of the [^{18}F]fluoride ion. In this study, for the same leaving group, the primary amides **1a** and **1b** gave just marginally lower RCYs of [^{18}F]*N*-fluoroacetyl-anilines than the secondary amides **1c** and **1d** (Table 1, cf. entries 13 with 8,

and 14 with 12). The conditions used here are probably insufficiently basic to depress the RCYs of the primary amides greatly.

Reduction conditions

The strong carbon–fluorine bond is generally quite resistant to chemical reducing agents, even strong reducing agents that readily reduce other carbon–halogen bonds.[49–51] Borane-THF was selected as a convenient reagent for the reduction of **4a** to [^{18}F]*N*-(2-fluoroethyl)-aniline **5a** *in situ*. Reduction was achieved by treating the crude reaction mixture from the nucleophilic substitution reaction with borane-THF under mild conditions (10 min, 65°C). The conversion of **4a** into **5a** was estimated to be 65–70% from HPLC analysis. NCA [^{18}F]*N*-(2-fluoroethyl)-aniline **5a** was produced in 40% RCY overall within 50 min of radionuclide production. The reaction proceeded cleanly. The only other radioactive species detected at the end of reaction was unreacted [^{18}F]fluoride ion. Similarly **4b** was reduced to **5b** in about 70% RCY (representing an overall RCY from [^{18}F]fluoride ion of about 40%). By-products identified by MS were the corresponding 2-hydroxy-*N*-methyl-*N*-phenyl-acetamide **7** and *N*-(2-bromoethyl)-*N*-methyl-aniline **8**. These were easily separable from the corresponding radioactive product (Figure 5).

A requirement of this method of [^{18}F]*N*-(2-fluoroethylation) is that the compound to be labeled should not contain other functional groups that might be easily and undesirably reduced by the selected reducing agent. For wider application of this method, other reducing agents might be considered for the reduction of the intermediate ^{18}F-labeled amide to the required [^{18}F]*N*-(2-fluoroethyl)-aniline, depending on the structure of the compound to be reduced.

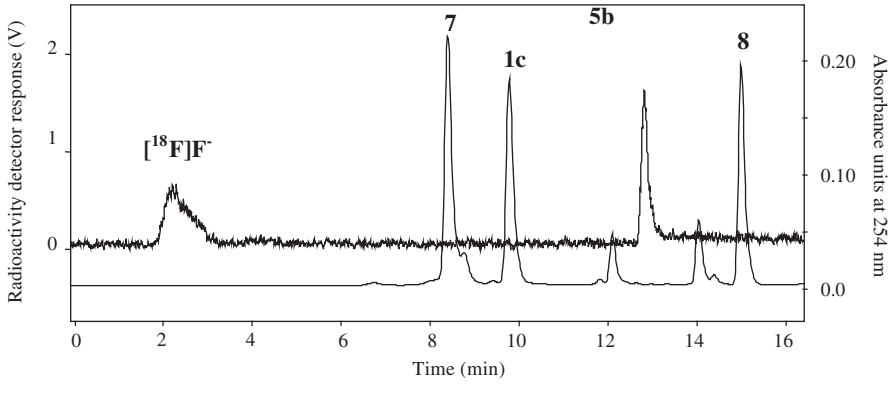

Figure 5. Representative chromatogram from the HPLC analysis of the reaction mixture from a reduction reaction (of 4b to 5b). 7: 2-hydroxy-*N*-methyl-*N*-phenyl-acetamide, 8: *N*-(2-bromoethyl)-*N*-methyl-aniline

Conclusions

The described two-step one-pot process provides an alternative route for the NCA [^{18}F]*N*-2-fluoroethylation at a weakly nucleophilic center (aromatic amine). The overall RCY is about 40% and acceptable for application to the radiosynthesis of PET radiopharmaceuticals. The preferred bromo precursors are easily prepared and stored. Inclusion of a fixed proportion of water in the first stage, the nucleophilic substitution reaction with NCA [^{18}F]fluoride ion in a glass vessel, affords high RCY and reproducibility.

Experimental

Materials

Ammonium formate (96%, J.T. Baker) and acetonitrile (high purity solvent, Burdick & Jackson) were used as supplied. Kryptofix 222® (4,7,13,16, 21,24-hexaoxa-1,10-diazabicyclo[8,8,8]hexacosane) (98 + %), 18-crown-6 (1,4, 7,10,13,16-hexaoxacyclooctadecane) (99.5 + %), 2-hydroxymethyl-18-crown-6 (95 + %), borane-THF complex (1 M in THF in Sure/Seal™ bottle), aniline (99.5 + %), *N*-methyl-aniline (99 + %), bromoacetic anhydride (99 + %), chloroacetic anhydride (97 + %), potassium carbonate (99.995%), potassium iodide (99 + %), potassium bicarbonate (ReagentPlus™), potassium fluoride (99.99 + %, anhydrous), THF (99.9 + %, inhibitor-free, anhydrous) and other common chemicals were purchased from Aldrich and used as received. Glass vessels (5 ml; borosilicate glass; tapered cone; fitted with phenolic caps and TFE/silicone liners; Mini-vial 20/400) for radiochemistry were purchased from Alltech (Deerfield, USA) and were cleaned in water and acetone, and then dried at 100°C before use.

General methods

Radioactivity was measured using a calibrated dose calibrator (Atomlab™ 300; Biodex Medical Systems) with correction for background and physical decay.

Each prepared compound was analyzed by high performance liquid chromatography (HPLC) performed on apparatus (System Gold; Beckman Coulter) equipped with a reverse phase column (C18; 10 µ particle size; 250 × 4.60 mm o.d.; Phenomenex) eluted with MeCN-0.01 M ammonium formate at 2 ml/min [gradient (v/v) from 20:80 to 70:30 over 12 min then isocratic (70:30) for 8 min]. The eluate was monitored for absorbance (254 nm; 166 detector, Beckman Coulter) and where appropriate simultaneously for radioactivity (diode or PM Flow-count detector; Bioscan). Each ^{18}F-labeled compound was identified by retention time (R_t) and co-injection of the corresponding reference compound.

RCYs of labeling reactions were estimated by analytical HPLC, with integration of radioactive peaks; they represent conversions, not isolated yields. The area of the product peak (P, in units of V min) was compared to the integral of radioactivity from all eluted peaks (T, in units of V min) and the radiochemical yield calculated as ($P/T \times 100\%$). No corrections were made for radioactive decay in the short time between the first and last eluted peak ($\sim$12 min). Regular checks were performed, confirming that all radioactivity injected onto HPLC was eluted during the course of analysis.

^{1}H- ^{13}C- and ^{19}F-NMR spectra were recorded on an Avance-400 spectrometer (Bruker). Chemical shifts in ^{1}H- and ^{13}C-NMR are reported in δ units (ppm) downfield relative to tetramethylsilane. Chemical shifts in ^{19}F-NMR are reported in δ units (ppm) downfield from fluorotrichloromethane as reference. Abbreviations s, d, dt, t and m denote singlet, doublet, double triplet, triplet and multiplet, respectively.

IR analysis was performed on a Spectrum One FT-IR spectrometer (Perkin Elmer).

Melting points are uncorrected and were determined with an Electrothermal apparatus (MEL-TEMP®).

LC-MS analysis was performed on a LC apparatus (Thermo Finnigan Surveyor), equipped with a reverse phase column (C18; 5 µm, 150 × 2 mm i.d.; Phenomenex), eluted with a gradient of MeOH-H_2O-0.5% AcOH at 0.15 ml/min, linked to electrospray MS (ThermoFinnigan LCQ_{DECA}). GC-MS analysis was performed on a Thermo Finnigan Polaris Q instrument equipped with a capillary column (Rtx-5MS 30 m × 0.25 mm i.d.; Restek). The carrier gas was helium (1 ml/min) and the oven temperature heated from 60 to 180°C at 20°C/min. The source temperature of MS was 250°C and the analysis performed in the electron ionization (+ve) mode.

*Synthesis of N-haloacetyl-anilines (**1a–1d**)*

General procedure: aniline or *N*-methyl-Aniline (20 mmol) was dissolved in glacial acetic acid (10 ml). As appropriate, bromoacetic anhydride or chloroacetic anhydride (20 mmol) was added dropwise to this solution at RT. The reaction mixture was stirred for 2 h at RT. Acetic acid was removed under reduced pressure. Aqueous NaOH (1M) was added to the residue and the product extracted with CH_2Cl_2 (3 × 10 ml). The organic layer was washed with cold water, dried over $MgSO_4$, filtered and concentrated. Thus prepared were:

N-bromoacetyl-aniline **1a** as slightly beige crystals (72%) after recrystallization (EtOH-H_2O). M.p.: 132°C. HPLC: $R_t = 9.1$ min. ^{1}H-NMR ($CDCl_3$) δ: 4.02 (s, 2 H), 7.17 (t, 1 H, $J = 7.4$ Hz), 7.36 (t, 2 H, $J = 7.5$ Hz), 7.53 (d, 2 H, $J = 7.5$ Hz), 8.20 (s, 1 H, NH); cf. Lit.[52] ^{13}C-NMR ($CDCl_3$) δ: 29.49, 120.04 (2C), 125.21,

129.12 (2C), 136.91, 163.32. GC-MS, $m/z = 214.06$ and 216.06 $[M+H]^+$ IR (KBr): 1660 cm^{-1}.

N-chloroacetyl-aniline **1b** as white crystals (74%) after recrystallization (EtOH-H_2O). M.p.: 130°C. HPLC: $R_t = 8.7$ min. ^{1}H-NMR ($CDCl_3$) δ: 4.20 (s, 2 H), 7.17 (t, 1 H, $J = 7.4$ Hz), 7.35 (t, 2 H, $J = 7.6$ Hz), 7.53 (d, 2 H, $J = 7.6$ Hz), 8.20 (s, 1 H, NH); cf. Lit.[34] ^{13}C-NMR ($CDCl_3$) δ: 42.89, 120.13 (2C), 125.26, 129.15 (2C), 136.68, 163.77. GC-MS, $m/z = 170.09$ and 172.09 $[M+H]^+$. IR (KBr): 1632 cm^{-1}.

N-bromoacetyl-N-methyl-aniline **1c** as slightly yellow crystals (78%) after recrystallization (EtOH-H_2O). M.p.: 47°C. HPLC: $R_t = 9.4$ min. ^{1}H-NMR ($CDCl_3$) δ: 3.30 (s, 3 H), 3.70 (s, 2 H), 7.26–7.32 (m, 2 H), 7.36–7.49 (m, 3 H); cf. Lit.[53] ^{13}C-NMR ($CDCl_3$) δ: 26.76, 38.10, 127.03 (2C), 128.55, 130.03 (2C), 143.09, 166.58. GC-MS, $m/z = 227.00$ and 229.02 $[M]^+$. IR (KBr): 1643 cm^{-1}.

N-chloroacetyl-N-methyl-aniline **1d** as slightly purple crystals (79%) after recrystallization (EtOH-H_2O). M.p.: 69°C. HPLC: $R_t = 9.1$ min. ^{1}H-NMR ($CDCl_3$) δ: 3.30 (s, 3 H), 3.85 (s, 2 H), 7.24–7.27 (m, 2 H), 7.36–7.50 (m, 3 H); cf. Lit.[53] ^{13}C-NMR ($CDCl_3$) δ: 38.01, 41.51, 127.09 (2C), 128.59, 130.09 (2C), 142.75, 166.30. GC-MS, $m/z = 183.05$ and 185.07 $[M]^+$. IR (KBr): 1687 cm^{-1}.

*Synthesis of N-fluoroacetyl-anilines (**2a** and **2b**)*

General procedure: A mixture of compound **1a** or **1c** (3.5 mmol), dry potassium fluoride (8.9 mmol), and di(ethylene glycol) (5 ml) was stirred and heated rapidly to 150°C and kept at this temperature for 2 h. The reaction mixture was cooled, diluted with water (10 ml), and extracted with toluene (3 × 10 ml). The organic layer was washed with sodium bicarbonate solution (5% w/v; 10 ml), dried over $MgSO_4$, filtered and concentrated. Thus, obtained were:

N-fluoroacetyl-aniline **2a** as white crystals (53%) after column chromatography (silica gel; hexane-AcOEt, 80:20 v/v). M.p.: 131°C; cf. Lit.[35] HPLC: $R_t = 7.4$ min. ^{1}H-NMR ($CDCl_3$) δ: 4.91 (d, 2 H, $J = 47.4$ Hz), 7.18 (t, 1 H, $J = 7.4$ Hz), 7.35 (t, 2 H, $J = 7.5$ Hz), 7.57 (d, 2 H, $J = 7.6$ Hz), 8.00 (s, 1 H, NH). ^{13}C-NMR ($CDCl_3$) δ: (80.41, 82.27, $J_{C-F} = 188$ Hz), 121.26 (2C), 126.28, 130.30 (2C), 137.58, 166.69. ^{19}F-NMR ($CFCl_3$) δ: −221.44. GC-MS, m/z = 154.12 $[M+H]^+$. IR (KBr): 1673 cm^{-1}.

N-fluoroacetyl-N-methyl-aniline **2b** as slightly beige crystals (55%) after chromatography (silica gel; hexane-AcOEt, 80:20 v/v): M.p.: 89°C; cf. Lit.[54] HPLC: $R_t = 7.5$ min. ^{1}H-NMR ($CDCl_3$) δ: 3.31 (s, 3 H), 4.65 (d, 2 H, $J = 47.2$ Hz), 7.18–7.26 (m, 2 H), 7.38–7.48 (m, 3 H). ^{13}C-NMR ($CDCl_3$) δ:

37.30, (77.67, 79.42, $J_{C-F}=176.1$ Hz), 126.98 (2C), 128.73, 130.16 (2C), 141.37, 166.76. ^{19}F-NMR ($CFCl_3$) δ: −225.01. GC-MS, $m/z=168.10$ $[M+H]^+$. IR (KBr): 1668 cm^{-1}.

*Synthesis of N-(2-fluoroethyl)-anilines (**3a** and **3b**)*

General procedure: A solution of BH_3 (1 M; 30 mmol) in THF was added dropwise for 1 h to a solution of the *N*-fluoroacetyl-aniline **2a** or **2b** (3 mmol) in anhydrous THF (3 ml) under N_2 at 0°C. The mixture was stirred for 30 min at 0°C and refluxed for 1 h. Excess BH_3 was quenched with methanol (3 ml) at 0°C and the solution evaporated to dryness. The oily residue was treated with a mixture of CH_2Cl_2 and brine. The organic layer was separated, dried over $MgSO_4$, filtered and concentrated. Thus prepared were:

N-(2-fluoroethyl)-aniline **3a** as an oil (71%) after column chromatography (silica gel; hexane, 0.5% NEt_3-AcOEt; 80:20 v/v). HPLC: $R_t=11.1$ min. ^{1}H-NMR ($CDCl_3$) δ: 3.40 (dt, 2 H, $J=26.5$, 4.9 Hz), 3.90 (s, 1 H, NH), 4.60 (dt, 2 H, $J=47.4$, 4.8 Hz), 6.63 (d, 2 H, $J=8.6$ Hz), 6.73 (t, 1 H, $J=7.3$ Hz), 7.18 (t, 2 H, $J=7.4$ Hz); cf. Lit.[55] ^{13}C-NMR ($CDCl_3$) δ: (44.13, 44.33, $J_{C-F}=20.5$ Hz), (81.64, 83.30, $J_{C-F}=167$ Hz), 113.16 (2C), 118.09, 129.38 (2C), 147.61. ^{19}F-NMR ($CFCl_3$) δ: −223.79. LC-MS, $m/z=140.10$ $[M+H]^+$.

N-(2-fluoroethyl)-N-methyl-aniline **3b** as an oil (68%) after column chromatography (silica gel; hexane, 0.5% NEt_3-AcOEt, 80:20 v/v). HPLC: $R_t=12.7$ min.^{1}H-NMR ($CDCl_3$) δ: 3.00 (s, 3 H), 3.64 (dt, 2 H, $J=24.0$, 5.2 Hz), 4.59 (dt, 2 H, $J=47.2$, 5.3 Hz), 6.67–6.76 (m, 3 H), 7.20–7.27 (m, 2 H); cf. Lit.[56] ^{13}C-NMR ($CDCl_3$) δ: 39.05, (52.68, 52.87, $J_{C-F}=21.4$ Hz), (81.0, 82.7, $J_{C-F}=169.8$ Hz), 112.37 (2C), 116.87, 129.29 (2C), 148.99. ^{19}F-NMR ($CFCl_3$) δ: −221.33. LC-MS, $m/z=154.10$ $[M+H]^+$.

NMR and LC-MS study of alkylation of Kryptofix 222 by N-2-bromoacetyl-N-phenyl-aniline

A solution of **1a** (2.3 mg; 10 μmol), Kryptofix 222 (3.9 mg; 10 μmol) and $KHCO_3$ (1.0 mg; 10 μmol) in CD_3CN (0.5 ml) was heated at 130°C in a sealed glass vial for 10 min. The following NMR signals were recorded from this mixture at the end of reaction: ^{1}H-NMR (CD_3CN) δ: 2.35 (m, 12 H, CH_2N), 3.36 (m, 12 H, CH_2O), 3.45 (s, 12 H, OCH_2CH_2O), 4.30 (s, 2 H), 7.25 (m, 4 H), 7.65 (m, 1 H). ^{13}C-NMR (CD_3CN) δ: 53.41, 67.15, 69.93, 125.33, 126.77, 128.77. LC-MS of the reaction mixture: $m/z=511.4$ $([M+1]^+$; calculated for $C_{26}H_{45}N_3O_7$, 510.3). Reference Kryptofix 222 gave the following NMR spectra: ^{1}H-NMR (CD_3CN) δ: 2.5 (t, 12 H, CH_2N), 3.46 (t, 12 H, CH_2O), 3.55 (s, 12 H, OCH_2CH_2O); ^{13}C-NMR (CD_3CN) δ: 55.70, 69.33, 70.11.

Radiochemistry

Radionuclide production. [^{18}F]Fluoride ion was produced by the $^{18}O(p,n)^{18}F$ reaction, by irradiation of ^{18}O-enriched water (95 atom %; 1.8 ml) with a beam of protons (14.1 MeV; 20–25 μA). Generally, this method produces [^{18}F]fluoride ion with a specific radioactivity exceeding 400 GBq/μmol. Portions (20–200 μl) of the whole irradiated water were used for individual experiments in this study.

Nucleophilic substitution reaction–general procedure. A glass reaction vial (5 ml; V-vial) was loaded with an aqueous solution (10 μl) of $KHCO_3$ (2.3 mg; 23 μmol) plus a solution of 18-crown-6 (6.2 mg; 23 μmol) in MeCN (0.15 ml). Cyclotron-produced NCA [^{18}F]fluoride ion (< 74 GBq) in [^{18}O]water (20–200 μl) was added and the solvent evaporated under reduced pressure (controlled with a bleed of nitrogen) while heating at 110°C (oil bath). Water was removed azeotropically during three cycles of addition-evaporation of MeCN (0.3 ml each cycle). A solution of H_2O (1 μl; 56 μmol) in MeCN (0.5 ml) was added to the dry residue, followed by addition of *N*-haloacetyl-aniline (3 mg; ~14 μmol) in MeCN (0.15 ml). The reaction vial was sealed, heated at 130°C for 10 min and then cooled to RT. The solution was then withdrawn. Radioactivities in solution and adhered to the reaction vessel were measured. The reaction solution was then analyzed by radio-HPLC. Reactions were repeated with altered parameters as indicated in Table 1.

Reduction–general procedure. The reaction mixture from the nucleophilic substitution reaction was cooled in a dry ice–acetone bath for 1 min, and then concentrated to 0.1 ml under reduced pressure at 20°C. The mixture was dissolved in THF (0.5 ml) containing water (1 μl). A solution of BH_3 in THF (1M; 0.1 ml) was then added. The reaction vial was heated at 65°C for 10 min and then cooled in a dry ice–acetone bath for 1 min. Excess BH_3 was then hydrolyzed with H_2O (1 ml). The reaction mixture was cooled and then analyzed by radio-HPLC.

Acknowledgements

The authors are grateful to Drs Frederick T. Chin and John L. Musachio (MIB, NIMH) for technical advice during this work, Dr Umesha Shetty (MIB, NIMH) for NMR and MS measurements, and Dr Shui-Yu Lu (MIB, NIMH) for critical discussion.

References

1. Phelps ME, Mazziotta JC. *Science* 1985; **228**: 799–809.
2. Phelps ME. *Neurochem Res* 1991; **16**: 929–940.
3. Phelps ME. *Proc Natl Acad Sci USA* 2000; **97**: 9226–9233.

4. Wagner HN. *J Nucl Med* 1991; **32**: 561–564.
5. PET for drug development and evaluation, Comar D (ed.). Kluwer Academic Publishers, Dordrecht, The Netherlands, *Dev Nucl Med* 1995; **26**: 1–384.
6. Guillaume M, Luxen A, Nebeling B, Argentini M, Clark JC, Pike VW. *Appl Radiat Isot* 1991; **42**: 749–762.
7. Ferrieri RA. In *Handbook of Radiopharmaceuticals*, Welch MJ, Redvanly CS (eds.), Ch. 7. John Wiley & Sons Ltd: Chichester, UK, 2003; 229–282.
8. Coenen HH, Colosimo M, Schuller M, Stöcklin G. *J Label Compd Radiopharm* 1986; **23**: 587–595.
9. Shiue CY, Bai L-Q, Teng R-R, Wolf AP. *J Label Compd Radiopharm* 1987; **24**: 55–65.
10. Block D, Coenen HH, Stöcklin G. *J Label Compd Radiopharm* 1987; **24**: 1029–1042.
11. Block D, Coenen HH, Stöcklin G. *J Label Compd Radiopharm* 1988; **25**: 201–215.
12. Chesis PL, Welch MJ. *Appl Radiat Isot* 1990; **41**: 259–265.
13. Oh S-J, Choe YS, Chi DY, Kim SE, Choi Y, Lee KH, Ha H-J, Kim B-T. *Appl Radiat Isot* 1999; **51**: 293–297.
14. Bergman J, Eskola O, Lehikoinen P, Solin O. *Appl Radiat Isot* 2001; **54**: 927–933.
15. Zheng L, Berridge MS. *Appl Radiat Isot* 2000; **52**: 55–61.
16. Comagic S, Piel M, Schirrmacher R, Höhnemann S, Rösch F. *Appl Radiat Isot* 2002; **56**: 847–851.
17. Zhang M-R, Tsuchiyama A, Haradahira T, Yoshida Y, Furutsuka K, Suzuki K. *Appl Radiat Isot* 2002; **57**: 335–342.
18. Iwata R, Pascali C, Bogni A, Furumoto S, Terasaki K, Yanai K. *Appl Radiat Isot* 2002; **57**: 347–352.
19. Zhang M-R, Furutsuka K, Yoshida Y, Suzuki K. *J Label Compd Radiopharm* 2003; **46**: 587–598.
20. Wilson AA, Da Silva JN, Houle S. *Appl Radiat Isot* 1995; **51**: 765–770.
21. Skaddam MB, Kilbourn MR, Snyder SE, Sherman PS, Desmond TJ, Frey KA. *J Med Chem* 2000; **43**: 4552–4562.
22. Chaly Jr T, Matacchieri R, Dahl R, Dhawan V, Eidelberg D. *Appl Radiat Isot* 1999; **51**: 299–305.
23. Block D, Coenen HH, Laufer P, Stöcklin G. *J Label Compd Radiopharm* 1986; **23**: 1042.
24. Coenen HH, Laufer P, Stöcklin G, Wienhard K, Pawlik G, Böcher-Schwarz, Heiss W-D. *Life Sci* 1987; **40**: 81–88.
25. Satyamurthy N, Barrio JR, Bida GT, Huang S-C, Mazziotta JC, Phelps ME. *Appl Radiat Isot* 1990; **41**: 113–129.
26. Goodman MM, Kilts CD, Keil R, Shi B, Martarello l, Xing D, Votaw J, Ely TD, Lambert P, Owens MJ, Camp VM, Malveaux E, Hoffman JM. *Nucl Med Biol* 2000; **27**: 1–12.
27. Davis MR, Votaw JR, Bremner JD, Byas-Smith MG, Faber TL, Voll RJ, Hoffman JM, Grafton ST, Kilts CD, Goodman MM. *J Nucl Med* 2003; **44**: 855–861.

28. Wester HJ, Herz M, Weber W, Heiss P, Senekowitsch-Schmidke R, Schwaiger M, Stöcklin G. *J Nucl Med* 1999; **40**: 205–212.
29. Piel M, Bauman R, Schirrmacher R, Rosch F. *J Label Compd Radiopharm* 2003; **46**: S207.
30. Chin FT, Musachio JL, Cai L, Pike VW. *J Label Compd Radiopharm* 2003; **46**: S172.
31. Loustau-Then I, Ponchant M, Fuseau C, Karmenka JM, Vignon J, Crouzel C. *Nucl Med Biol* 1997; **24**: 513–518.
32. Mulholland GK, Jung YW, Wieland DM, Kilbourn MR, Kuhl DE. *J Label Compd Radiopharm* 1993; **33**: 583–591.
33. Chi DY, Kilbourn MR, Katzenellenbogen JA, Welch MJ. *J Org Chem* 1987, **52**: 658–664.
34. Akturk Z, Filic F, Erol K, Pabuccuoglu V. *Il Farmaco* 2002; **57**: 201–206.
35. Shahak I, Bergmann ED. *J Chem Soc (C)* 1967: 319–320.
36. Baillet S, Buisine E, Horvath D, Maes L, Bonnet B, Sergheraert C. *Bioorg Med Chem* 1996; **4**: 891–899.
37. Hamacher K, Coenen HH, Stöcklin G. *J Nucl Med* 1986; **27**: 235–238.
38. Liotta CL, Harris HP. *J Am Chem Soc* 1974; **96**: 2250–2252.
39. Albanese D, Landini D, Penso M. *J Org Chem* 1998; **63**: 9587–9589.
40. Landini D, Maia A, Rampoldi A. *J Org Chem* 1989; **54**: 328–332.
41. Conant JB, Kirner WR, Hussey RE. *J Am Chem Soc* 1925; **47**: 488–501.
42. Bordwell FG, Brannen Jr WT. *J Am Chem Soc* 1964; **86**: 4645–4650.
43. Kilbourn MR, Brodack JW, Chi D, Dence CS, Jerabek PA, Katzenellenbogen JA, Patrick TB, Welch MJ. *J Label Compd Radiopharm* 1986; **23**: 1174–1176.
44. Korguth ML, DeGrado TR, Holden JE. *J Label Compd. Radiopharm* 1988; **25**: 369–380.
45. Brodack JW, Kilbourn MR, Welch MJ, Katzenellenbogen JA. *Appl Radiat Isot* 1986; **37**: 217–221.
46. Feller D. *J Phys Chem* 1997; **101**: 2723–2731.
47. Krongsuk S, Kerdcharoen T, Hannongbua S. *J Phys Chem B* 2003; **107**: 4175–4181, and references therein.
48. Rogers GA, Stone-Elander S, Ingvar M, Eriksson L, Parsons SM, Widèn L. *Nucl Med Biol* 1994; **21**: 219–230.
49. Gambaretto G, Conte L, Fornasieri G, Zarantonello C, Tonei D, Sassi A, Bertani R. *J Fluor Chem* 2003; **121**: 57–63.
50. Choe YS, Lidstrom PJ, Chi DY, Bonasera TA, Welch MJ, Katzenellenbogen JA. *J Med Chem* 1995; **38**: 816–825.
51. Hendrix JA, Stefany DW. *Tetrahedron Lett* 1999; **40**: 6749–6752.
52. Zucchi G, Scopelliti R, Buenzli JC. *J Chem Soc Dalton Trans* 2001; **13**: 1975–1985.
53. Diehl K, Himbert G, Henn L. *Chem Ber* 1986; **119**: 2430–2443.
54. Kano S, Taniguchi K. *Japan Pat* 1966; 14,987. *Chem Abstr* 1966; **65**: 20055e.
55. Wimalesema K, May SW. *J Am Chem Soc* 1987, **109**: 4036–4046.
56. Fuchigami T, Ichikawa S. *J Org Chem* 1994; **59**: 607–615.

